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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 5 AUGUST, 1947 No. 8

HOW AND WHY A BETTER USE OF FOOD

The how and the why of the changing of food habits are closely interwoven. People of whatever age and background are best persuaded to improve their food habits—their habitual decisions as to what kinds of foods to eat and how much of each—not by dogmatic pronouncements of what "is good for you," but by giving to all teaching of food values the dignity and interest of a participation in a scientific advance. As we make clear what recent research has discovered about the relations of food to nutrition and of nutrition to health, we make people *want* the benefits which come from better food habits.

The ambitions thus aroused and enhanced in individuals, families, and community groups by nutrition teaching are reflected (a) as consumer demand upon producers and purveyors of food, and (b) as public opinion stimulating and upholding the efforts of all educational and governmental agencies in behalf of nutritional guidance of all food affairs.

That our people can be induced to change their food habits on a nationwide scale, though gradually, is shown by the fact that they have done so (F. Clark, B. Friend, and M. C. Burk, "Nutritive Value of the Per Capita Food Supply, 1909–45," *a processed publication of the U. S. Dept. Agriculture June (1946)*).

Between 1915 and 1945 our national yearly per capita consumption of milk increased 52 per cent; of green and yellow vegetables, 79 per cent; of citrus fruits and tomatoes, 133 per cent.

Thus in thirty years—the conventional one generation of human affairs—our people increased their per capita consumption of milk 52, that of green and yellow vegetables 79, per cent. These are substantial changes in food habits, and doubtless predominantly due to the increase and diffusion of nutrition knowledge. In the case of citrus fruits and tomatoes the same educational influences were at work together with the economic developments which changed the status of oranges and grapefruit from luxuries to staple foods, and here per capita consumption was more than doubled in a generation.

Hence substantial changes of food habits are within the realm of practical facts.

Precisely how one may best formulate a statement of the benefits which result from better use of food will obviously depend upon the age, education, and presumed nutritional status of those whom we are addressing. In the educational efforts inaugurated by the National Nutrition Conference held in Washington in May, 1941, effective use has been made of the concept of three goals. These were, and are: (a) Complete eradication of nutritional deficiency diseases—"we do not have yellow fever any longer in this country, neither should we have pellagra"; (b) Great reduction of those infectious diseases which like tuberculosis are effected greatly by nutritional status; and (c) Such levels of nutritional status for all as to ensure to each that degree of "health plus" that is potential in his hereditary birthright.

This threefold grouping of the health benefits of a better use of food is a device for convenience of thinking and discussion, and does not imply that the three goals are entirely separate or that they are successive in the sense of a time sequence. Rather we should endeavor and expect that every nutritional-education project and every "action program" for the betterment of food supply conditions will benefit its population (whether that of a village or a nation) in all three ways at the same time. Any suggestion of a sequence among the three

goals is chiefly an order of obviousness: the most obvious first and the most ambitious last.

In the United States our national food crops, if equitably distributed, would provide amply adequate dietaries for all of our people. How to bring about such equitable distribution is both an educational and an economic problem. Taking account both of incomes and means of growing a part of the family food at home, the great majority of American families are economically able to provide themselves amply adequate dietaries when educated to make full use of nutritional knowledge in the guidance of their expenditures of money and time. In this sense the problem may be said to be predominantly educational; but it should also be said that the influence of nutritional education may affect expenditures for other things as well as for foods.

It is probably neither necessary nor practicable to separate fully and clearly the educational and the economic aspects of the problem. Both are involved, for example, in the already widespread project of school feeding, which as carried on in progressive localities is both a part of the educational work of the school and an "action program" for the cure and prevention of malnutrition among children of school age.

The improvements of food habits brought about through school feeding, and through the teaching of food values and nutrition in schools of all grades and directly to the public, have undoubtedly helped toward the attainment of all three of the above-mentioned goals, whether or not the improvements can yet be measured in health statistics. Inasmuch as the diagnosis of malnutrition is still a subject of active research, we must expect delay in the correlation of causes and effects in the enhancement of health through improvement of food habits. Yet when we consider together the findings of direct human experience and those of animal experimentation, the evidence as a whole is so convincing as clearly to call for prompt and steady teaching of the practicability and significance of beneficial changes in food habits. Such adjustments as are nutritionally most desirable can all be made without the exclusion of any kind or type of food from the dietary, but rather by shifting the relative proportions of some types of foods in the light of our increased knowledge of nutrition.

Coordination of the guidance (a) of habits of food consumption, and (b) of such food production programs as shall meet consumer demands in the markets, is greatly facilitated by the bracketing of foods into about ten groups as the writer has recently explained elsewhere ("Foods: Their Values and Management," *Columbia University Press, December (1946)*). For the immediate purpose of the present brief paper, we may simplify still further into six food groups as follows: (1) the grain crops; (2) the vegetables and fruits; (3) milk and its products other than butter; (4) meats, fish, poultry, and eggs; (5) fats; and (6) sugar and sweets.

If the dietary or food habit is approached openmindedly and from the viewpoint of making the fullest use consistent with present nutritional knowledge of each of these food groups in the order here given, the following far-reaching potentialities of such readjustment of food habits become clear.

The increased prominence which would thus be given to fruits, vegetables, and milk in the food budgets of typical American consumers will undoubtedly improve their nutritional status. Correspondingly it will tend to moderate the demands of American consumers upon the meat, fat, and sugar supplies, thus making economically feasible a more equitable distribution of these latter types of food among the world's people,—with great gain to good will and the sense of social justice. And this latter can at the same time be further promoted if the relatively high-income people will specifically moderate their market demands for highly grain-fed meats; because the production of

these means the feeding to meat animals of large amounts of grain, of which a more substantial share should, in the interest of human nutrition, be turned to increased production of bread (so long as there are people in want of bread) and to permanently increased milk production.

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PRESENT KNOWLEDGE OF RIBOFLAVIN IN NUTRITION

The existence of the water soluble, yellow-green, fluorescent pigment in whey, now known as riboflavin, was recognized as early as 1879 but without any knowledge of its unusual importance in the nutriture of respiring tissues (A. W. Blyth, *J. Chem. Soc.* **35,** *530 (1879)*). For fifty years, concentration of this pigment and its striking chemical properties were studied with little progress. In 1932 and 1933, reports of the isolation of this material in pure form from natural materials and its significance as an essential growth factor for laboratory animals and as an integral constituent of some oxidation enzyme systems were published independently by several laboratories (O. Warburg and W. Christian, *Biochem. Z.* **254,** *438 (1932)*; P. Ellinger and W. Koschara, *Ber. d. deutsch. chem. Gesellsch.* **66,** *315, 808 (1933)*; R. Kuhn, P. György, and T. Wagner-Jauregg, *Ibid.* **66,** *317, 576 (1933)*). The characterization and synthesis of the riboflavin molecule were soon reported (R. Kuhn, K. Reinemund, F. Weygand, and R. Ströbele, *Ibid.* **68,** *1765 (1935)* and P. Karrer *et al., Helvet. chim. acta* **18,** *1435 (1935)*).

Riboflavin is widely distributed throughout the plant and animal kingdoms with its rich sources in anaerobic, growing, fermenting bacteria. Milk, liver, kidney, and heart are excellent sources, many vegetables are also good sources, but the cereals are rather low. The riboflavin concentration in oats, wheat, barley, and corn is increased strikingly during germination (*Nutrition Reviews* **1,** *356 (1943)*). In natural material where there is little respiration or fermentation, riboflavin is found to a greater extent in the free state than in combined form, for example, in milk (*Ibid.* **4,** *134 (1946)*), in urine (A. Emmerie, *Acta brevia Neerland. Physiol., Pharmacol., Microbiol.* **8,** *116 (1938)*), and in the retina (E. Adler and H. von Euler, *Nature* **141,** *790 (1938)*). However in tissues where respiration is taking place, riboflavin is predominantly present as its phosphoric acid ester and as the riboflavin-phosphoric acid-adenine-dinucleotide and in combination with specific proteins. Liver slices from riboflavin deficient animals have a decreased ability for oxidizing d-amino acids but the *in vitro* addition of riboflavin dinucleotide returns the capacity to a normal level. The activity of xanthine oxidase and succinate dehydrogenase was likewise reduced (*Nutrition Reviews* **3,** *291 (1945)*). These enzymes and several others, including Warburg's "yellow enzyme," contain riboflavin (*Ibid.* **5,** *194 (1947)*).

Riboflavin is relatively stable in ordinary cooking procedures with a retention of 70 to 85 per cent in the meat itself after roasting, braising, or boiling. An additional 15 per cent of the original riboflavin was recovered from the drippings (*Nutrition Reviews* **1,** *281 (1943)*; **2,** *6 (1944)*; **4,** *61 (1946)*). The losses of riboflavin in milk during pasteurization, irradiation to enrich with vitamin D, and exposure to light during bottling are in the neighborhood of 10 to 20 per cent. The destruction of riboflavin in milk by exposure to light after bottling may be extensive unless proper precautions to protect bottles from light are taken (*Ibid.* **3,** *126 (1945)*). When stored in vacuum tins, dehydrated meat samples maintained almost the original concentration of riboflavin even when stored

up to or at 99°F. for ten months (*Ibid.* **2,** *336* (*1944*)).

A rather wide variety of manifestations of riboflavin deficiency have been reported in experimental animals: degeneration of nerve tissue in rats (*Nutrition Reviews* **1,** *45* (*1942*)), reduced hemoglobin formation in rats (*Ibid.* **1,** *357* (*1943*); **2,** *235* (*1944*)), congenital malformation in rats (*Ibid.* **2,** *297* (*1944*)), dermatitis, diarrhea, incoordination, and nerve degeneration in swine (*Ibid.* **3,** *141* (*1945*)), and a cessation of the estrus cycle in the rat (*Ibid.* **4,** *71* (*1946*)). In the very young calf fed a "synthetic milk" which contained no riboflavin, a definite ariboflavinosis has been produced. This condition was characterized by hyperemia of the buccal mucosa, lesions in the corners of the mouth and along the edges of the lips, loss of appetite, poor growth, scours, excessive salivation and lacrimation but without vascularization of the cornea or opacity of the lens (A. C. Wiese, B. C. Johnson, H. H. Mitchell, and W. B. Nevens, *J. Nutrition* **33,** *263* (*1947*)). A substantial synthesis of riboflavin has been demonstrated in the rumen of steer with artificial fistulas (*Nutrition Reviews* **1,** *297* (*1943*)). In the experiment with young calves, the bacterial flora of the rumen had not been established sufficiently to permit adequate riboflavin synthesis to supply the requirements of these growing animals.

In addition to the above evidences of experimental ariboflavinosis, several other associations have been observed in riboflavin deficient animals. For example, the count of parasitized red blood cells after injection of Plasmodium lophurae in riboflavin deficient chicks became only one fifth of that observed in chicks receiving an adequate amount of riboflavin. Riboflavin deficient rats have been shown to have a lower resistance to endemic typhus than their controls. Riboflavin deficient mice succumb more rapidly to the intraperitoneal injection of virulent pneumococci than normal ones (*Nutrition Reviews* **3,** *69* (*1945*)). A low riboflavin intake results in an increased incidence of the type of tumors produced by feeding butter yellow (p-dimethylaminoazobenzene) which apparently causes an increased excretion of riboflavin and decreased tissue concentrations (*Ibid.* **1,** *247* (*1943*)).

The riboflavin concentration of most tumors was found to be relatively constant between 2 and 3 micrograms per gram of moist tissue, which is well within the range for normal tissues (*Nutrition Reviews* **1,** *271* (*1943*)). The livers of riboflavin deficient rats had a greatly reduced ability to inactivate estradiol (*Ibid.* **3,** *27* (*1945*); **5,** *26* (*1947*)). The livers of animals fed a diet low in protein had lower riboflavin concentrations than those of animals with normal protein intake (*Ibid.* **1,** *267* (*1943*)). The livers of the protein deficient animals likewise had a reduced ability to inactivate estradiol which was not entirely due to the reduced riboflavin concentrations (*Ibid.* **3,** *27* (*1945*)). The protein content of riboflavin deficient chicks has been shown to be less than that of pair fed controls (*Ibid.* **1,** *397* (*1945*)).

W. H. Sebrell and R. E. Butler in 1938 (*Pub. Health Reports* **53,** *2282* (*1938*)) reported the experimental production of riboflavin deficiency in man. Ten of the 18 women maintained on the riboflavin deficient diet developed macerated areas in the corners of the lips which progressed to transverse fissuring (cheilosis). The labial mucosa became shiny, abnormally red, and denuded; greasy, seborrheic accumulations were observed around the nose, eyes, and in some cases on the ears. Oral administration of synthetic crystalline riboflavin resulted in a relief of these signs. Ocular manifestations of human ariboflavinosis have been described by H. D. Kruse *et al.* (*Nutrition Reviews* **1,** *194* (*1943*)). The tongue in cases of human riboflavin deficiency is magenta or purplish-red in color and painful with a burning sensation (*Ibid.* **1,** *116* (*1943*)); vulval lesions have also been reported (*Ibid.* **4,** *319* (*1946*)). A discussion of the value of these signs in the diagnosis of human

ariboflavinosis and of the complicated and conflicting etiology of these lesions has been presented (*Ibid.* **4,** *130 (1946)*).

In another experimental riboflavin deficiency in man, the subjects were apparently in excellent health after three months on the riboflavin deficient regimen. The urinary excretion of riboflavin fell for two weeks and then remained constant at 150 to 250 micrograms per day; fecal excretion became relatively constant at 200 to 600 micrograms per day (*Nutrition Reviews* **3,** *81 (1945)*). The combined excreta thus contained five to six times as much riboflavin as was ingested in the food. Administration of succinylsulfathiazole in the above experiment did not reduce the riboflavin output appreciably but did reduce the fecal excretion of thiamine. Later experiments on the utilization of dietary riboflavin and the excretion of riboflavin have been described (*Ibid.* **5,** *60 (1947)*). A minimum requirement of 0.5 mg. of riboflavin per 1000 calories for adult women was observed in one of these experiments. The figure recently suggested in the Recommended Dietary Allowances of the Food and Nutrition Board for moderately active adult women was 0.6 to 0.7 mg. per 1000 calories with an increase to 1.0 mg. per 1000 calories during pregnancy and lactation (*Ibid.* **3,** *287 (1945)*). Studies on the utilization of riboflavin during lactation indicated that satisfactory lactation was possible on a diet supplying 1.0 mg. of riboflavin per 1000 calories of food consumed (*Ibid.* **5,** *107 (1947)*).

RELATIONSHIP OF GASTRIC ACIDITY TO THIAMINE DEFICIENCY IN THE AGED

Observations on experimental animals and in man have yielded conflicting data concerning the effect of thiamine deficiency upon the gastric secretion. Thus, the work of G. R. Cowgill and A. Gilman (*Arch. Int. Med.* **53,** *58 (1934)*) suggested that thiamine deficiency suppresses the gastric secretion. The observations of some investigators confirmed these conclusions but those of others disagreed with this view. It is of interest that in a report bearing on this point, R. D. Williams, H. L. Mason, and B. F. Smith (*Proc. Staff Meetings Mayo Clinic* **14,** *787 (1939)*) noted a low level or an absence of acidity in 4 young women maintained on a low thiamine diet.

H. A. Rafsky and B. Newman (*Am. J. Med. Sci.* **201,** *749 (1941)*; **205,** *209 (1943)*) have made several reports on the nutritional status of the aged, including data on ascorbic acid and niacin requirements, as well as thiamine excretion (*Gastroenterology* **1,** *737 (1943)*). In the last mentioned report they presented evidence suggesting a certain degree of thiamine "unsaturation" in aged persons as judged by failure to reach a constant excretion of thiamine in the urine until substantial amounts of thiamine had been given.

In a later paper H. A. Rafsky, B. Newman, and N. Jolliffe (*J. Lab. Clin. Med.* **32,** *118 (1947)*) report results of thiamine excretion tests and gastric analyses in 31 persons (14 men and 17 women) ranging from 65 to 81 years of age. All were apparently normal and were being fed an institutional diet adequate in thiamine (0.51 to 1.11 mg. per day) as well as in other vitamins.

Twenty-four hour urinary thiamine excretion was measured. In this series of subjects 45 per cent were found to excrete less than 50 micrograms of thiamine per day, an amount regarded as subnormal. A similar number showed low hydrochloric acid secretion, including 6 subjects with complete lack of secretion of hydrochloric acid even following the injection of histamine. There was no significant correlation, however, between gastric anacidity or hyposecretion and urinary thiamine excretion.

It does not follow from this report that there is no relationship between gastric secretory response and thiamine deficiency. Even if it is admitted that low urinary thiamine excretion indicates thiamine deficiency, it is probable that this is not an index of severe deficiency. It is likely that impairment or inhibition of gastric secretion is the result of thiamine deficiency only when the deficiency is severe.

NUTRITION SURVEY IN CANADA

In Canada, a nutrition survey including physical examination, dietary studies, and blood analyses has been carried out in British Columbia and Saskatchewan (L. B. Pett and F. W. Hanley, *Canad. Med. Assn. J.* **56,** *187 (1947)*). The studies were made in cooperation with provincial boards of health, Red Cross Society, and local personnel.

In British Columbia 1495 pupils in sixteen different schools and, in addition, 157 Chinese and 26 Sikh children were examined. Most of the children were from 5 to 11 years of age, the sample constituting about 1.7 per cent of the children of this age group in the province. Dietary records, physical examinations, and blood testing were carried out by the usual methods. There had been no idea of the kind or amount of malnutrition in Canada and this report is one of the first of a series which, it is hoped, will give a reasonable picture of the nutritional problem among younger schoolchildren.

The results were tabulated in the usual manner. Separate tables on dietary intake, hemoglobin, serum proteins, concentration of ascorbic acid, and on the incidence of various single signs of deficiency states are given. Since few diseases have a single pathognomonic sign, the authors felt that two or more signs in one individual greatly increases the probability that the person has a specific nutritional disorder. A list of signs encountered in cases of various dietary deficiencies is given, although many of these were not encountered in any of the subjects studied.

An earnest effort was made to correlate the diet history, signs, and laboratory findings, as is done in other branches of medicine, so as to yield an accurate diagnosis of "definite" malnutrition. This group was not large. A slightly larger group, labeled "probable deficiencies," included cases showing dietary inadequacies of less than half the usual recommendations, as well as those showing perhaps one physical sign of a deficiency, but a good diet history or a high blood level. The authors refuse to diagnose malnutrition exclusively on a poor dietary record, on the basis that suitable standards are not available, but point out that many such people must ultimately be undernourished. Similarly the presence of one of the many unspecific signs sought in nutrition studies, is not accepted as evidence even of "probable" deficiency unless accompanied by another sign or by a poor dietary record over a period of time, or by a low biochemical test of some kind. This method is similar to that used by J. Metcoff *et al.* (*Nutrition Reviews* **3,** *309 (1945)*) in a survey carried out in Newfoundland and marks an advance in the diagnosis of mild malnutrition over the older methods of listing individual signs. Therapeutic tests would be a valuable addition to this procedure.

No great effort was made to give a statistical evaluation. One child out of 12 studied in British Columbia was found to be anemic. The values for serum ascorbic acid in this province averaged 0.57 mg. per 100 ml.; about 40 per cent of the values were below 0.4 mg. per 100 ml. The protein values averaged 6.9 g. per 100 ml. of serum. Edema was not found, but in 18 per cent of the group values for serum protein were

less than 6.0 g. The most common manifestation of malnutrition was underweight which was noted in 14 per cent of the subjects. It can be assumed that the thin child is not getting enough food for reserve of energy in work and play or protection in disease and injury. Definite evidence that the child had had rickets was found in 11 per cent of the group; this seemed consistent with the fact that few children examined ever took fish liver oil.

Anemia was noted in only 3 per cent of the group studied in Saskatchewan. The average concentration of ascorbic acid was 0.43 mg. per 100 ml. and it was less than 0.4 mg. per 100 ml. in more than 56 per cent of the group. Average value for serum protein was 6.8 g. and in only 4 per cent was it less than 6 g. Eleven per cent of the children were underweight and 5 per cent had definite evidence that they had had rickets.

Scurvy, beriberi, starvation, and other marked deficiency diseases in acute forms were not encountered in either province although a recently compiled table of deaths in Canada showed that 42 persons died of nutritional deficiencies in 1944. It is interesting for comparison that 38 deaths resulted from poliomyelitis in the same year.

Although riboflavin deficiency was not common in either province it was suspected that about one child in 5 had this deficiency. It was believed that this was accounted for by the low intake of milk. Thiamine deficiency was not noted, nor was an attempt made to measure it. The incidence of definite vitamin A deficiency varied from 0.5 to 0.8 per cent of the groups studied.

Since malnutrition may leave permanent disability in a certain percentage of cases and undoubtedly accounts for some ill health and impaired working ability in others, such studies as these should be encouraged.

BIOTIN ANALOGUES AND MICROBIAL GROWTH

Compounds structurally related to biotin have been prepared in a number of laboratories. These substances affect the growth of microorganisms in three ways. Some possess biotin activity, some are physiologically inert, while still others inhibit the action of biotin in a competitive manner, suggesting that the analogue enters into a key position in the cell, presumably at the surface of an enzyme, and thus prevents the vitamin from fulfilling its usual role in metabolism.

Two reviews (*Nutrition Reviews* **3,** *36* (*1945*); **4,** *159* (*1946*)) have dealt with the earliest of these compounds to be prepared, notably desthiobiotin, biotin sulfone, imidazolidone caproic acid, imidazolidone valeric acid, and oxybiotin (O-heterobiotin). The list has now been extended to include homologues of oxybiotin in which the number of carbon atoms in the aliphatic side-chain is varied from 3 to 7 (K. Hofmann, C. Chen, A. Bridgwater, and A. E. Axelrod, *J. Am. Chem. Soc.* **69,** *191* (*1947*)). On the basis of the results obtained by these workers, together with those previously reported, some speculation is possible regarding the mode of attachment of biotin within the cell, as well as a temporary designation of the groups within the molecule which engage in metabolic activity.

The high activity of O-heterobiotin (25 to 50 per cent of biotin) is suggested by S. H. Rubin, D. Flower, F. Rosen, and L. Drekter (*Arch. Biochem.* **8,** *79* (*1945*)) to be due to conversion of the oxygen analogue to biotin during the growth of microorganisms. Since the analogue is easier to prepare than the vitamin, Hofmann and co-workers investigated the various analogues described above with the assumption that the biologic activities would par-

allel those of the corresponding biotin derivatives.

The activity of all of the oxybiotin homologues was very low, less than 0.0035 per cent that of biotin. Another compound, in which a carboxyl group was substituted for hydrogen alpha to the carboxyl group of oxybiotin (thus forming a substituted malonic acid) was about 3 per cent active. The authors conclude that the extreme side-chain specificity is due to the exact requirements for distance between the carboxyl group and the urea residue, a statement which seems in accord with the facts.

On the other hand, the various homologues appeared able to form at least a partial attachment within the cell. The 4-, 7-, and 6-carbon side-chain compounds were able to inhibit oxybiotin in yeast when the molar ratios of $\frac{\text{analogue}}{\text{oxybiotin}}$ were 143,000, 30,000 and 7400 respectively. The 3-carbon side-chain showed no inhibition, indicating little or no attachment. None of the analogues was able to inhibit biotin activity, however. This suggests that the attachment of the analogues is either too feeble to compete with biotin or that biotin is oriented toward the protein in a slightly different manner from oxybiotin.

A previous review (*Nutrition Reviews* **4,** *159 (1946)*) raised the interesting point that the conversion of oxybiotin to biotin during growth suggests the alternate opening and closing of the sulfur-containing ring during metabolism. An example of this type of action is found with thiamine; the disulfide formed is also analogous to cystine or the dimerized form of glutathione. That this concept may be correct is indicated by the extreme antibiotin activity of biotin sulfone (inhibition ratio = 280:1) which differs from biotin only in the state of oxidation of the sulfur atom. Likewise, removal of the sulfur atom produces a compound (desthiobiotin) which inhibits biotin in Lactobacillus casei at a ratio of 9100:1. (In yeast, desthiobiotin is 100 per cent active; this is probably due to resynthesis to biotin.) Complete removal of the sulfur ring leaves a substance (imidazolidone caproic acid) which is still capable of attachment within the cell; its inhibition ratios are 760,000:1 and 126,000:1 with yeast and L. casei, respectively.

The position of the two nitrogen atoms in the imidazole ring also seems important. The diamino carboxylic acid derived from biotin by hydrolysis is still 10 per cent active for yeast, as is the corresponding desthiobiotin derivative. The former compound is also active for L. casei (J. L. Stokes and M. Gunness, *J. Biol. Chem.* **157,** *121 (1945)*). The complete nitrogen ring seems necessary for combination with avidin, however (K. Dittmer and V. du Vigneaud, *Science* **100,** *129 (1944)*), so it is possible that the ring is synthesized from the diamino acids by the organisms.

The present results suggest that biotin is normally attached in the cell either through its carboxyl group or the heterocyclic nitrogen atoms, or both; and that the major portion of the metabolic activity is effected through the sulfur atom. A more complete elucidation of the role of the active centers in the biotin molecule would require testing compounds in which (1) the carboxyl group and (2) the heterocyclic nitrogen atoms are altered or covered in such a manner as to prevent combination with other substances. The production of biologically inert material by this means could be taken to indicate that the group in question constitutes a point of attachment within the cell; an active antimetabolite, on the other hand, should be capable of attachment but incapable of metabolic activity.

NIACIN DEFICIENCY IN SWINE

The intriguing relationship between niacin and tryptophane which has been demonstrated in rats and in dogs (*Nutrition Reviews* **4,** *182, 334 (1946)*) probably plays an equally important role in swine. Although the nutritional requirements of this species have been uncertain (*Ibid.* **3,** *79 (1945)*) there appears to be no doubt that swine pellagra occurs and that niacin is an essential nutrient except possibly in animals fed rations rich in protein. Thus, no evidence of specific niacin deficiency was observed in pigs supplied a food mixture containing 26 per cent of casein whereas typical signs appeared if casein was fed at a 10 per cent level (M. M. Wintrobe, H. J. Stein, R. H. Follis, and S. Humphreys, *J. Nutrition* **30,** *395 (1945)*). That this protein effect was probably due to the tryptophane of casein is suggested by the antipellagric action of supplements of either niacin or of tryptophane in young pigs fed low protein diets (R. W. Luecke, W. N. McMillen, F. Thorp, and C. Tull, *Ibid.* **33,** *251 (1947)*). In these experiments corn or oats were the principal dietary constituents and total protein was varied by the use of small amounts of casein and of soybean oil meal. A ration containing corn 80, casein 12, soybean oil meal 6, salt mixture 2, and vitamin supplements, including 30 mg. of niacin daily, permitted good growth during a six to eight week period. The pigs were healthy in appearance and showed no gross pathology on autopsy. Omission of niacin resulted in poorer growth and 3 of 5 animals developed a mild diarrhea within four weeks. Autopsy disclosed a thickening of the large intestine with severe congestion of the mucous membrane. If the total protein was decreased from 19 to 14 per cent by substitution of corn for part of the casein and soybean oil meal, growth was very poor, the hair coat appeared rough, the skin around the ears was scaly, and 4 of 5 pigs developed severe diarrhea. Autopsy revealed intestinal lesions which were more marked than those observed on the higher protein diet. Daily administration of 30 mg. of niacin to a similar group of pigs on the low protein regimen resulted in animals of normal appearance although the growth rate was retarded. No intestinal abnormalities were observed. Daily supplementation of another group with 200 mg. of dl-tryptophane produced sleek healthy-appearing animals with a rate of growth superior to that of the group receiving the niacin supplement. However, autopsy revealed a few colonic lesions in 2 of 5 pigs. The lesions were not severe. Pigs supplied an oat diet (15.9 per cent protein) grew poorly and were untidy but did not exhibit diarrhea or intestinal lesions. The colons were distended with oat hulls and the authors ascribed the retarded growth to the high fiber content of this ration.

During the first and last experimental weeks, twenty-four hour urines were collected and analyzed for thiamine, riboflavin, pantothenic acid, niacin, N′-methylnicotinamide, and tryptophane. Estimates of total intakes of these nutrients were not included in the paper but it is stated that the excretion of thiamine and of riboflavin represented only a small proportion of the extra supplements provided daily. One-half or more of the supplement of calcium pantothenate appeared in the urine. Very little niacin was excreted, the amount being less than 6 per cent of the intake in the pigs receiving the largest quantity. The excretion of N′-methylnicotinamide was lowest on the low protein corn diet and approximately the same increases resulted from supplementation of the diet with niacin (30 mg.) or with dl-tryptophane (200 mg.). The greatest excretion of the methyl derivative occurred on the high protein corn ration supplemented with niacin. This ration also furnished the most

dietary tryptophane. Only 1 per cent or less of the intake of this amino acid was excreted, the quantity varying with the level in the diet.

Diarrhea and intestinal inflammation occur in swine on diets deficient in pantothenic acid (*Nutrition Reviews* **3,** *269* (*1945*)), but Luecke *et al.* do not believe that pantothenic acid was involved in their experiments. It is possible that the observed intestinal pathology is related to necrotic enteritis of swine (*Ibid.* **2,** *14* (*1944*)), although bacteriologic examination of the inflamed colons failed to reveal the presence of pathogenic organisms. Though administration of tryptophane was only partially protective, the improved condition of the pigs receiving either the amino acid or the vitamin supplement justified the conclusion that the inadequacy of the low protein, corn diet was due to a deficiency of niacin and that the latter was in some way spared, replaced, or increased as a result of the presence of extra tryptophane. These experiments support the view that the requirement of niacin in the pig, as in the rat and dog, is increased if the intake of tryptophane is low. The relation of the utilization of tryptophane in niacin deficiency to its utilization as an amino acid in normal protein metabolism is not apparent at the present time. The determination of the exact influence of varying levels of dietary niacin on the daily requirement of tryptophane may aid in the clarification of this problem.

METABOLIC RELATIONS OF SOME DERIVATIVES OF NIACIN

The trigonelline which occurs in normal urine arises from the ingestion of the preformed substance in foodstuffs. It is no longer believed that trigonelline is an end product of niacin metabolism in man (*Nutrition Reviews* **3,** *263* (*1945*)), and it has not been generally held that trigonelline is converted to niacin by man. P. P. Foā and A. S. Glicksman (*Arch. Biochem.* **11,** *477* (*1946*)) have reinvestigated their earlier observation (H. Field, P. P. Foā, and N. L. Foā, *Ibid.* **9,** *45* (*1946*)) that the ingestion of trigonelline was followed by the excretion of an increased amount of niacin-like substances in the urine as determined by the Melnick-Field procedure for niacin. Five healthy subjects were maintained on a low niacin diet and three consecutive eight hour samples of urine were collected. An oral dose of 300 mg. of trigonelline was then administered and the urine was collected for the succeeding two days. The free and total niacin excretion was estimated by the USP XII microbiologic assay procedure. No significant increase in the urinary excretion of niacin was found following the oral administration of 300 mg. of trigonelline. This report confirms the earlier experiences of H. P. Sarett, J. W. Huff, and W. A. Perlzweig (*J. Nutrition* **23,** *23* (*1942*)) that the ingested trigonelline is excreted quantitatively and does not increase the urinary output of niacin.

Much evidence is accumulating that tryptophane may serve as a precursor of niacin in the rat (*Nutrition Reviews* **4,** *334* (*1946*)). Studies of this relationship in the horse, which does not require niacin, and the cotton rat, a species requiring niacin, have been reported by B. S. Schweigert, P. B. Pearson, and M. C. Wilkening (*Arch. Biochem.* **12,** *139* (*1947*)). In these studies Shetland ponies were maintained on a diet consisting of dried beet pulp, corn, casein, salts, and vitamin supplements which provided approximately 9.0 mg. of niacin and 3 g. of tryptophane per day. Urinary collections were made during a two day period on the basal diet alone, following which one group of ponies received 6 g. of dl-tryptophane per day for a three day period and another group received 3 g.

of dl-tryptophane daily for three days. Urinary collections were made on the second and third days of supplementation and for two additional days after tryptophane feeding was discontinued. The urine was analyzed for N′-methylnicotinamide by a fluorometric procedure, for niacin by a microbiologic assay, and for "apparent free tryptophane" by a microbiologic technic. The average excretion of niacin rose from 1.8 mg. per twenty-four hours on the basal diet alone to 6.9 mg. per twenty-four hours during the fourth to sixth day. In the group of ponies receiving 6 g. of dl-tryptophane per day, on the seventh day the average niacin excretion was 4.4 mg. per twenty-four hours. In this group of animals the N′-methylnicotinamide excretion altered from 3.6 mg. per twenty-four hours on the basal diet alone to 4.4 mg. per twenty-four hours during the period of supplementation with tryptophane and 5.9 mg. per twenty-four hours on the seventh day after the supplementation had been terminated. In the 3 animals receiving 3 g. of tryptophane per day as supplements there was no significant increase in niacin excretion in the urine. These slight stimulatory effects of ingested tryptophane on the niacin excretion in the horse are in distinct contrast to the pronounced effects observed when tryptophane is fed to rats. One of the more striking differences between these two species is that in the horse there occurred no augmentation of N′-methylnicotinamide excretion after ingestion of the amino acid.

Two groups of 4 cotton rats each were maintained on Purina dog chow and urine collections were made for a three day period. dl-Tryptophane was then added to the basal diet in quantities of 100 mg. per day and urine collections continued for an additional three day period. The excretion of niacin and N′-methylnicotinamide was determined on the pooled specimens for the two periods. The excretion of N′-methylnicotinamide during the period on the basal diet alone averaged 167 and 86 mg. per rat per day by the two groups. During the period of supplementation this excretion rose to 518 and 282 micrograms per rat per day respectively for the two groups. This effect of tryptophane is much less than has been reported for the albino rat. Such comparative studies on other species may aid in the elucidation of the problem of the metabolic relationship of the pellagra-preventive substances.

AMINO ACID REQUIREMENTS FOR MAINTENANCE OF ADULT RATS

The requirements of amino acids for growing rats have been widely studied, but requirements for maintenance of adult animals have been less thoroughly investigated. The latter is of interest, however, because of clinical concern with maintenance of nitrogen balance by means of parenteral amino acid mixtures. The determination of the preformed amino acids required for maintenance of nitrogen equilibrium appears to be a justified criterion since it has been demonstrated that "endogenous catabolism" of nitrogen can be neither depressed nor stimulated by feeding amino acids or proteins (E. W. Burroughs, H. S. Burroughs, and H. H. Mitchell, *J. Nutrition* **19,** *271 (1940)*).

This principle has been employed in a study of the amino acids required by the adult rat for maintenance (*J. Nutrition* **19,** *363 (1940)*). In these experiments, adult female albino rats were fed a standardizing diet for a period of at least twelve to fourteen days. The ration contained about 5 per cent of protein and an abundance of the B-vitamins. After this standardizing period the animals were placed on a nitrogen free diet of the following composition: starch 63, sucrose 10, salt mixture (modified Osborne and Mendel) 5, lard 10, butterfat

10, cod liver oil 1.5, and wheat germ oil 0.5. An additional 1.0 or 2.0 g. of sucrose was given daily to each animal depending upon the ability of the rat to maintain its weight. Beginning on the second day of this regimen each rat was supplied a complete amino acid mixture equivalent to 80.0 mg. of nitrogen daily and this mixture was fed until positive and uniform nitrogen balances were obtained. This ordinarily required five to ten days. The nitrogen in this amino acid mixture was equally divided among the following amino acids: dl-threonine, dl-isoleucine, l-tryptophane, dl-valine, dl-methionine, dl-lysine, l-histidine, dl-phenylalanine, dl-leucine, d-arginine, l-cystine, l-tyrosine, glycine, dl-alanine, dl-serine, dl-norleucine, dl-aspartic acid, dl-glutamic acid, l-proline, and l-hydroxyproline.

To determine the effect of a given amino acid on the nitrogen balance each amino acid was withdrawn individually from the mixture; the incomplete mixture was then fed at the rate of 80.0 mg. of nitrogen per rat per day. The period of deficient feeding was continued until uniform daily nitrogen balances again occurred. This period was usually three to five days in length. The rats were next returned to the complete amino acid mixture and again this period was continued until consistent, positive nitrogen balances were evident. If positive nitrogen balance did not result in this latter period the experiment was discarded. Throughout these three periods each animal was force-fed its entire ration twice daily, thus insuring ingestion of equal quantities of diet by each rat.

The experimental results indicated that the amino acids necessary for the replacement of the loss of nitrogen in the "endogenous catabolism" of the adult rat were: threonine, isoleucine, tryptophane, valine, methionine, phenylalanine or tyrosine, and leucine or norleucine. It was concluded that the adult rat does not require the following dietary amino acids for the replacement of endogenous losses of nitrogen: lysine, leucine, histidine, arginine, and phenylalanine. It is to be recalled that all of these amino acids are required for growth in the rat. They further concluded that the adult rat requires both cystine and methionine for the maintenance of nitrogen equilibrium and, as in the process of growth, the cystine requirement can be met by dietary methionine but the converse is not true. Either tyrosine or phenylalanine could serve to meet the maintenance requirement of the adult animal for the aromatic amino acids. Since there is little evidence that tyrosine is converted to phenylalanine in the animal organism these investigators concluded that the endogenous metabolism of the adult rat involves the destruction of tyrosine, but not the destruction of phenylalanine. Finally, they concluded that the adult rat may be maintained in nitrogen equilibrium on a diet containing only the following amino acids: threonine, isoleucine, tryptophane, valine, methionine, tyrosine, and norleucine.

Studies which at first sight seem to fail to support the conclusions of Burroughs, Burroughs, and Mitchell (*loc. cit.*) have been reported by A. Neuberger and T. A. Webster (*Biochem. J.* **39,** *200 (1945)*) who investigated the lysine requirement of the adult rat maintained on a diet in which the amino acids were supplied as zein and tryptophane. The food was given ad libitum and the experimental period was of several weeks duration. The addition of lysine to the diet was necessary for weight maintenance throughout the period. They noted a marked disparity, however, between the maintenance requirements and the growth requirements of rats for lysine. The minimum maintenance requirements were set by these workers at 16.0 mg. per day, the requirements for growth were estimated at 100.0 mg. per day. Close scrutiny of the details of these two series of experiments reveals that they are not strictly comparable and that the varied observations are prob-

ably reconcilable. It is widely recognized that on a low protein intake a decreased caloric consumption aggravates the protein deficiency. The experiments of Mitchell and co-workers were over short periods with adequate caloric intake assured and nitrogen provided from a varied group of amino acids. These conditions are optimal for the definition of a minimal list of amino acids indispensable for maintenance of the adult animal. Over longer periods of time in which the caloric intake was restricted, it does not appear illogical that maintenance requirements might exceed the synthetic ability of the organism for certain other amino acids.

H. H. Mitchell (*Arch. Biochem.* **12,** *293 (1947)*) has again emphasized the pronounced difference between the lysine requirement of the rat for growth and for maintenance. He reports that the net utilization in per cent of food proteins fed at a 10 per cent level to the growing rat were as follows: rolled oats 61, white flour 52, whole wheat 61, and cereal mixture 60. For adult rats the net utilization of the protein of these cereals fed at 4 to 5 per cent protein levels was 78, 62, 69, and 75 respectively. This increased efficiency of utilization by the adult rat is interpreted to indicate differences in the amino acid requirement between the growing and the adult rat. Since cereals are generally deficient in lysine, it is suggested from these data that the requirement of the rat for lysine either disappears entirely when growth is complete or assumes an inconspicuous role. In other experiments in which lysine was added as the supplement to a diet of white bread, the results were indecisive. Mitchell concluded that for the adult rat lysine is "either a dispensable dietary component, or the requirement for it is very small indeed in comparison with that for other amino acids."

In an effort to account for the rat having a less stringent requirement for lysine, histidine, and arginine for maintenance than does the adult man, Mitchell sets forth an hypothesis of more than usual interest. He suggests that during growth some if not all of the amino acids essential for growth in the rat are synthesized in the tissues, but at rates which are inadequate to meet the requirements during the period of growth. At the completion of this period, however, the synthetic reactions may be able to meet the demands for some of these amino acids for maintenance. In the rapidly growing rat the synthetic reaction to growth may be proceeding at an intense rate and when growth is completed there is a great decrease in the need for the synthesis. Therefore, it is suggested that "the residual capacity to synthesize cellular elements, including the 'essential amino acids,' may be adequate to cover the requirements for some of them, such as arginine, histidine, lysine, leucine and phenylalanine, for the replacement of endogenous losses. In such cases, the dietary requirement disappears." If valid, this hypothesis may be of interest in relation to other dietary essentials in addition to the amino acids. At any rate it should indicate the difficulty of employing data on the requirements of one species during growth to estimate maintenance requirements of that or another species during adult life.

RELATION OF INHERITANCE TO NUTRITIONAL REQUIREMENT

The distinction is made often, and perhaps too easily, that the determination of a biologic event is due to (a) environment, or (b) heredity, or as Francis Galton once put it "to Nurture or to Nature." The utility of a science of nutrition is predicated on

the base that the nutrition of an organism is part of its environment and hence an understanding of the facts of nutrition leads facilely to a control of biologic events in which the organism participates. There is, however, an intuitive recognition that certain biologic events are completely under the control of heredity, while environment, including nutrition, plays no part in their determination, e.g., the blood groups, to take an example of medico-legal standing.

Even those items of the nutritional environment which are effective in their influence of certain biologic events must operate in a genotype, and if genotypes vary, as they certainly do, then it must be anticipated that at some time the growing science of nutrition must come to grips with the problem of genotype. This is well recognized right now at the species level and is recognition of the fundamental reason why, for example, ascorbic acid as an item of diet has greater meaning for guinea pigs, monkeys, and man than it does for the rat or the mouse. At the intraspecific level, however, information is very meager. As the science of nutrition expands we may expect increasing attention to the limits that the genetic composition of an organism puts on the effect of nutrition, or put another way, to the diversity of the effects of a nutrient as it operates in diverse genetic circumstances. Some beginnings in this direction have been made.

H. P. Morris, L. S. Palmer, and C. Kennedy (*Minn. Agr. Exp. Sta. Tech. Bull.* **92** (*1933*)) studied the relation of inheritance to efficiency of food utilization in the rat. They found that there were significantly different efficiency indexes for the two sexes with the average female efficiency index being 1.7 times that of the male during the six week period of most rapid growth. There was also a wide variation in food utilization within the members of each sex. By selective breeding two lines of rats were established for different rates of food utilization in which the lower efficiency strain was about 40 per cent less efficient than the rats of the high efficiency line. J. W. Gowen (*Genetics* **21,** *1* (*1936*)) observed a wide difference in the survival of different strains of rats fed the rachitogenic diet of Steenbock and Black. The variation in life expectancy for males on this deficient regimen was between 132 ± 6 days and 344 ± 15 days, and for females was between 116 ± 6 days and 274 ± 9 days. The strains which exhibited greater survival for male rats showed greater survival for females; strains which exhibited shorter survival for males also showed shorter survival for females. Males on the rachitogenic diet lived slightly longer than the females but the reverse was true of the animals maintained on the control diet.

Three different strains of white rats were maintained on diets which contained different levels of thiamine (R. F. Light and L. J. Cracas, *Science* **87,** *90* (*1938*)). When fed a thiamine deficient diet supplemented with 2 micrograms of thiamine per rat per day, the rats of the strain which required the least thiamine grew an average of 33.3 ± 1.9 g. during the five week test period; in contrast the rats of the strain requiring the highest amount of thiamine grew an average of only 14.0 ± 1.6 g. in response to the same supplement. When rats of the former strain were given 4 micrograms of thiamine per day they grew an average of 52.8 ± 2.3 g., while the latter strain required 8 micrograms of thiamine per day to gain a similar amount of weight during the same experimental period. R. W. Engel (*Proc. Soc. Exp. Biol. Med.* **52,** *281* (*1943*)) selected rats which were susceptible or resistant to choline deficiency. Both sexes of the F_2 generation of these two groups of rats had strikingly different choline requirements with much higher incidences of kidney hemorrhage and of fatalities among the strain with the high choline requirement than among the low choline requirement strain. The most strik-

ing differences were observed in the females with an incidence of kidney hemorrhages in 19.7 per cent of the low choline strain in contrast to 93.9 per cent of the high choline strain. The percentage of fatalities was 1.5 and 42.4, respectively, for the two groups.

W. F. Lamoreux and F. B. Hutt (*Genetics* **28,** *79 (1943)*) developed strains of White Leghorn chicks which are relatively resistant and others which are relatively susceptible to riboflavin deficiency. Resistance was measured by the ability to survive and to gain weight during the first te weeks of life while receiving a diet deficient in riboflavin. The gain in weight was 50 per cent greater for chicks of the resistant strain than for those in the susceptible line. During the same period deaths in the resistant strain were less than one third of those in the susceptible line.

Other manifestations of varying responses to the same diet by different representatives of the same species have been described. In dental caries investigations, different strains of white rats have been observed to have different susceptibilities to the development of carious lesions. The development of strains of widely different caries resistance and susceptibilities have been produced by selective inbreeding (H. R. Hunt, C. A. Hoppert, and W. G. Erwin, *J. Dental Research* **23,** *385 (1944)*). Likewise different strains of hamsters (*Nutrition Reviews* **1,** *205 (1943)*) and different strains of cotton rats have been found to have significantly different caries susceptibilities (*Ibid.* **4,** *73 (1946)*).

H. R. Bird, M. Rubin, and A. C. Groschke (*J. Nutrition* **33,** *319 (1947)*) described an inborn characteristic in poultry which was manifested in the hatchability of eggs and the viability and growth of the chicks from hens fed a basal ration which contained no animal protein. The hens used in these experiments were yearling crossbreds (Rhode Island Red x Barred Rock) and were mated to New Hampshire males. They were fed a diet consisting of: ground yellow corn, alfalfa meal, soybean meal, butyl fermentation solubles, limestone flour, steamed bone meal, sodium chloride, manganese sulfate, and vitamins A and D in oil. Individual hatchability records were calculated over a ten month period for 183 hens housed on concrete floors covered with litter; in addition individual records were kept over a six month period for 67 hens housed in laying pens on raised wire floors. A number of hens in each group maintained excellent hatchability records during these observation periods, although the low hatchability of the eggs laid by the majority of the hens indicated that the diet was inadequate for these hens. There was essentially no difference between the hatchability records of the two groups of hens housed under different conditions. This would indicate that the coprophagy permitted in the group on the litter-covered floors had not supplemented the diet to any detectable extent. The distribution of hens in the first group with respect to the percentage of fertile eggs which hatched was as follows: 44 per cent of the hens laid eggs which had a hatchability between 0 and 70 per cent, 37 per cent whose eggs had a hatchability of 70 to 85 per cent, and 19 per cent with hatchability records between 85 to 100 per cent. Since these hens were continued on the basal ration for an additional six months without any significant alteration in the average hatchability records, this variability did not seem to be due to differences in the storage of the factor which determined the hatchability of the eggs or in differences in adaptability to the unfavorable ration. Likewise the efficiency of feed utilization and the rate of passage of feed through the digestive tract did not prove to be different among these hens. It is presumed that the observed differences in hatchability were genetic in origin. Breeding studies will be required for proof.

M. Rubin and H. R. Bird (*J. Biol. Chem.* **163,** *393 (1946)*) had shown that cow manure

and various concentrates prepared from it stimulated the growth of chickens which had been hatched from eggs laid by hens maintained on the above basal diet. Similarly, M. Rubin, H. R. Bird, and I. Rothchild (*Poultry Sci.* **25,** *526 (1946)*) observed that there was a growth promoting factor in the feces of hens. Urine-free feces were as effective as cow manure but the total excrement was less effective presumably due to the presence of urinary products. In the present study coprophagy did not play any apparent role in supplying the growth factor; it was thought that some of the hatchability differences observed between individual hens on the basal diet might have been due to the extent of synthesis of the deficient factor in the digestive tracts of the various hens. However feeding of 5 per cent manure from either the hens of low or high hatchability records to day-old chicks as supplements to the basal ration did not produce differing growth responses or significant growth increases in either case. However, the urine-free feces of a hen fed the basal diet and the total excrement of hens fed the control diet produced significant growth responses. Therefore these data would indicate that this growth factor was synthesized to some extent by hens maintained on the basal diet but the amounts present in the total excrement were not adequate to produce growth stimuli under the conditions of these experiments.

When chicks hatched from eggs laid by hens consuming this deficient ration were maintained for six weeks on the same ration, their growth was much slower than that of chicks whose dams had been fed a positive control diet. In addition, the progeny of hens whose fertile eggs had the highest hatchability rate grew appreciably faster than the chicks whose dams were in the low hatchability groups. The progeny of the former group had a viability which was in excess of the latter groups and compared favorably with those chicks from hens which had been fed a control ration.

H. R. Bird and M. Rubin (*Poultry Sci.* **25,** *87 (1946)*) have shown previously that supplements of rather high levels of calcium pantothenate and choline chloride to the basal ration permitted more rapid growth. Supplements of dl-methionine also had a stimulatory effect upon the growth of chicks maintained on the basal ration (H. R. Bird and J. P. Mattingly, *Ibid.* **24,** *29 (1945)*). However evidence has been presented which indicated that the concentrates prepared from cow manure did not owe their effect to any of these substances (Rubin and Bird, *loc. cit.*) In the present studies, when a concentrate of manure was given as a supplement the growth rates of the chicks from lines of varying hatchability were about the same even though the growth curves of the controls followed the hatchability trend. However, the growth stimulus of dl-methionine, calcium pantothenate, and choline chloride supplements was irregular with excellent responses in some cases and poor in others. The authors suggested that the unknown factor in manure, dl-methionine, and the combination of choline chloride and calcium pantothenate were to some extent interchangeable for the chicks from hens of high hatchability records but were not or were less interchangeable for the chicks from hens of lower hatchability records.

These varied examples of relations of inheritance to nutritional requirements demonstrate clearly the difficulties which may arise in the evaluation of the requirements of various nutrients for a given species. The necessity for making allowances for individual variations in a human population becomes more obvious when such data are considered. From an experimental standpoint these data pose the question of the description of genotypes suitable for nutritional experiments. H. A. Schneider (*Vitamins and Hormones* **IV,** *35 (1946)*) in a review of the relation of nutrition to resistance to infection has suggested that plasticity of a genotype to nutritional factors is a function of genetic heterogeneity.

In his view genetic homogeneity reduces this plasticity. In programs of inbreeding which have been followed through many generations in the albino rat and the mouse, many genotypes of each have been developed which vary greatly in constitution between one another and the original wild species. Thus, H. A. Schneider (*Proc. Soc. Exp. Biol. Med.* **63,** *161 (1946)*) found the wild genotype of house mouse to require certain environmental items such as light periodicity of certain duration, and opportunity for exercise, before breeding could be accomplished in the laboratory. Laboratory genotypes did not require these. Many interesting comparisons of laboratory genotypes with the wild genotype probably could be made with respect to nutritional requirements for each of our common laboratory animals.

THIAMINE DEFICIENCY AND THE PYRUVATE EXCHANGE OF THE HEART

Thiamine is necessary for the utilization of carbohydrate, this vitamin being part of a coenzyme in the oxidation of pyruvate. In thiamine deficiency, pyruvate accumulates and so also does lactate, frequently in a definite ratio to pyruvate (A. Chesler, E. Homburger, and H. E. Himwich, *J. Biol. Chem.* **153,** *219 (1944)*).

F. S. Randles, W. A. Himwich, E. Homburger, and H. E. Himwich (*Am. Heart J.* **33,** *341 (1947)*) became interested in determining whether the impaired ability of the body to utilize pyruvate in the presence of thiamine deficiency is a uniform abnormality in all tissues and whether this impairment in metabolism may be more marked in the heart. Two groups of dogs were used, the first being maintained on an adequate diet and the second being fed a mixture containing all known elements required to form a complete diet with the exception of thiamine. In the latter group the cardiac metabolism was examined in different stages of depletion. The experimental procedure consisted in anesthetizing the dog with pentobarbital and cannulating the trachea. The animal was then placed on artificial respiration, the sternum was split, and the sack covering the heart was slit to expose the heart. Blood samples were then drawn simultaneously from the coronary sinus, the structure into which most of the veins of the heart empty, and the femoral artery. The blood from the former site was assumed to give an indication of the metabolism of the heart muscle itself while the femoral artery blood gave information concerning the general metabolism of the body. Pyruvate, sugar, and lactate were measured by appropriate methods. Differences between the coronary sinus and femoral artery blood were regarded as significant when they were equal to or greater than 4 mg. per cent of glucose, 1.1 mg. per cent of lactate, and 0.12 mg. per cent of pyruvate. The data which are recorded indicate whether the differences were positive or negative or showed no significant change.

In normal animals, in most instances the values for lactate and especially for pyruvate were higher in the arterial blood than in the venous blood or showed no significant difference, while the glucose values varied in either direction. In animals in which thiamine deficiency was well marked, as indicated by the presence of neurologic signs, the pyruvate level in the coronary sinus blood was greater than in the femoral artery blood in eighteen out of twenty-six instances and no significant change was noted in seven. The lactate levels, on the other hand, were definitely higher in the femoral artery blood in fifteen out of twenty-four instances while the glucose levels were evenly distributed between positive, negative, and no change. In animals on the deficient diet in which neurologic signs had

not yet developed the levels of pyruvate and lactate were consistently higher in the femoral artery blood than in the coronary sinus while the glucose again was fairly evenly distributed. When the data on all the animals were averaged, it was found that in 13 normal animals the level of pyruvate in the coronary sinus was 0.66 mg. lower than in the femoral artery blood and in 11 deficient animals without neurologic signs it was 0.82 mg. lower. On the other hand, the average in 19 animals with neurologic signs was 0.47 mg. higher in the coronary sinus blood than in that from the femoral artery. It is of interest that in 5 dogs exhibiting pyruvate levels higher in the heart blood than in the peripheral blood, changes in the electrocardiogram were observed.

The pyruvate balance of the heart appears to be unlike what the same investigators have observed in the liver, intestine, and skeletal muscle. They conclude that normally the heart not only oxidizes all the carbohydrate split products formed within it but also utilizes some poured into the blood stream by other organs. In thiamine deficiency this function is impaired. It is suggested that an important source of the increased pyruvic acid blood levels observed in thiamine deficiency is the heart itself.

These observations are interesting and stimulating and deserve elaboration. It has been shown clearly that the heart is affected comparatively early by the metabolic defect associated with thiamine deficiency and striking alterations in function have been observed not only in man ("wet beriberi") but also in well controlled experiments in animals (M. M. Wintrobe, R. Alcayaga, S. Humphreys, and R. H. Follis, *Bull. Johns Hopkins Hosp.* **73,** *169 (1943)*). One would wish, however, that the data which Randles and associates record had been presented in more detail and that the difference in pyruvic acid, lactate, and glucose levels of venous and arterial blood taken, for example, from the femoral vein as well as the femoral artery had been reported. So many factors are known to influence the pyruvic acid blood level that one is left with some doubt about the reliability of differences of 0.12 mg. and comparisons of average values.

UTILIZATION OF d(+) TYROSINE BY MAN

The standard excretion technic used by A. A. Albanese and co-workers to study the utilization of d-amino acids by man has been described in an earlier article (*Nutrition Reviews* **4,** *173 (1946)*). This method appears to be particularly well suited to the study of d (+) tyrosine, because tyrosine is not indispensable for the growth (M. Womack and W. C. Rose, *J. Biol. Chem.* **107,** *449 (1934)*) or for the maintenance of the nitrogen equilibrium of man (W. C. Rose, W. J. Haines, and J. E. Johnson, *Ibid.* **146,** *683 (1942)*), hence these experimental methods cannot be employed with tyrosine.

The excretion of tyrosine and degradation products following administration of 0.01 mole of d (+)-, l (−)-, and dl-tyrosine to each of 4 human subjects have therefore been measured by A. A. Albanese, V. Irby, and M. Lein (*J. Biol. Chem.* **166,** *513 (1946)*). The amount of urinary tyrosine arising from ingestion of the d variety of tyrosine can be calculated from the tyrosine output for the seven hour period following administration of racemic and naturally occurring tyrosine, respectively. For the 4 subjects tested, feeding of 0.01 mole of dl-tyrosine caused an increase in tyrosine output equivalent to 12.0 to 18.0 per cent of the d component fed.

Since it is supposed that the intermediary

metabolism of tyrosine involves a fission of the benzene ring and subsequent formation of keto fatty acids, the determination of total organic acids excreted by the subjects was also made in the hope of obtaining additional data susceptible of interpretation.

The readings obtained disclosed that ingestion of dl-tyrosine unlike l(−)-tyrosine, causes an elevation in organic acid output above the normal levels. It appeared that, apart from the smaller fraction excreted as unchanged tyrosine, nearly all of the orally administered d(+)-tyrosine was degraded to aliphatic acids, and little if any became available to the body for normal physiologic functions.

Adding to this the observations of A. R. Cushny ("The Biological Relations of Optically Isomeric Substances," *pp. 46–47, Williams and Wilkins Co., Baltimore (1926)*) that natural levorotatory epinephrine is twice as strong a vasoconstrictor as the racemate, and of G. L. Foster, W. W. Palmer, and J. P. Leland (*J. Biol. Chem.* **115,** *467 (1936)*) that l-thyroxine has a calorigenic effect on normal guinea pigs approximately twice as great as that of racemic thyroxine, it seems reasonable to draw the general conclusion that mammals are not able to metabolize d-hydroxyphenolic substances in the same manner as the l-compounds.

EFFECT OF DIET ON STABILITY OF BODY FAT

There is support for the belief that the rancidity of foods, not infrequently encountered in poultry, bacon, pork, and fish, is conditioned by the result of diet which has altered the fatty acid composition and antioxidant content of the tissues. C. H. Lea (*J. Soc. Chem. Ind.* **50,** *343T (1931)*; "Rancidity in Edible Fats," *Chemical Publishing Co., New York (1939)*) showed that the body fat of hogs was increasingly susceptible to oxidation if cod liver oil was included in their feed, and it was suggested that diet might also affect the antioxidant content of body fat.

This general subject has been reviewed by G. O. Burr, W. O. Lundberg, and J. R. Chipault (*Oil and Soap* **23,** *382 (1946)*). Burr and co-workers, in a series of studies (R. H. Barnes, W. O. Lundberg, H. T. Hanson, and G. O. Burr, *J. Biol. Chem.* **149,** *313 (1943)*; W. O. Lundberg, R. H. Barnes, M. Clausen, and G. O. Burr, *Ibid.* **153,** *265 (1944)*; H. T. Hanson, R. H. Barnes, W. O. Lundberg, and G. O. Burr, *Ibid.* **156,** *673 (1944)*) measured the stability of abdominal fat of young albino rats kept on various diets for thirty to one hundred and fifty days by measuring in Warburg flasks, the increase in peroxide value during oxygen absorption. From these studies it appeared that the keeping qualities of the body fat of rats were not affected by the protein level or by adding foods thought to be rich in antioxidants. In the purified diet the type of fat was important and butterfat was more effective in increasing the stability of body fat than lard. Feeding wheat germ oil and hydroquinone had deleterious effects on the stability of the body fat, but tocopherols greatly improved stability. Alpha- and beta-tocopherol were nearly twice as effective as the gamma form, although the latter is several times more active than the others when added directly to rendered body fat.

More recently the fatty acid composition, tocopherol content, and keeping time of several samples of fat taken from the fresh carcasses of 2 hogs have been measured. When the tocopherol content is constant, the keeping time decreases with an increase in the linoleic content; with the latter constant, keeping time increases with an increase of tocopherol content.

Butterfat is greatly affected by diet in

addition to the seasonal variations in carotene and vitamin A which are well recognized. Flavors due to oxidation in milk and butterfat are said to be reduced by the addition of various natural antioxidants to the diet. In an experiment to test this, the vitamin E-free basal diet of one cow was supplemented with 6.2 g. of synthetic alpha-tocopherol each week, while a control animal received only the basal diet. The butterfat from these 2 animals was tested in the Warburg microrespirometers at 100°C. The induction period of the vitamin E-free butterfat was essentially zero while that of the butterfat from the cow receiving vitamin E was rather markedly increased.

There is thus little doubt that the keeping quality of food fats is influenced by the diet of the animal from which they are derived, and that this factor must receive attention in food marketing.

INFECTION AND IRON METABOLISM

The anemia associated with chronic infection is so commonplace but so rarely severe that relatively few precise observations of the morphologic characteristics and etiology have been made. It has usually been classed as an anemia due to toxic inhibition of the blood-forming organs. Important contributions to the understanding of this anemia, as well as to the study of anemia in general, have been made (G. E. Cartwright *et al.*, *J. Clin. Invest.* **25,** *65, 81 (1946)*; M. M. Wintrobe *et al.*, *Ibid.* **26,** *103, 114, 121 (1947)*). Chronic infections in man and induced infections in dogs were studied.

A review of the literature failed to give a clear picture of the characteristics of the anemia of chronic infection in man, although in previous work evidence of increased blood destruction was not found. Serum iron had been reported reduced in acute and chronic infections and massive iron therapy repeatedly shown to be ineffective.

The morphologic characteristics of the blood in 16 cases of the anemia accompanying infection revealed either normocytosis or microcytosis. No instance of macrocytic anemia was observed. The bone marrow revealed an increase in the ratio of myeloid cells to nucleated red blood cells. Megaloblasts were not observed but polychromatic and orthochromatic normoblasts predominated.

The plasma iron content was strikingly reduced in each of 19 cases of from one to thirty-six months' duration. The normal range was 120 ± 30 micrograms per cent. Hypoferremia developed rapidly in several cases of acute infection although none developed anemia. Ferrous sulfate by mouth (1.0 g.) failed to raise the plasma iron concentration in contrast to an almost threefold rise in 8 normals. Although the plasma iron rose in the patients following intravenous administration of iron ascorbate, the peak was lower and the duration of the elevated values much shorter in 6 patients than in 6 normals. Similar curves to those in chronic infection were found in one patient with hypochromic anemia.

The serum copper concentration was elevated in all cases of chronic infection studied and the erythrocyte protoporphyrin in all but one. The rise in erythrocyte protoporphyrin developed somewhat later than the fall in plasma iron and returned to normal more slowly, and was not observed in cases of acute infection with hypoferremia. Urinary coproporphyrin excretion was also increased in all patients studied, the determination being made at the height of the anemia. The serum bilirubin and plasma proteins were normal or occasionally in the case of bilirubin, slightly reduced.

Various therapeutic measures were tried but were ineffective in alleviating the

anemia. These included ferrous sulfate orally, iron ascorbate intravenously, copper sulfate orally, vitamin B-complex orally, penicillin intramuscularly, "Amigen" intravenously, globin, cystine, methionine, and crude extract of liver (Wilson) orally. Transfusions of whole blood were associated with a rise in the hematocrit, but failed to affect the hypoferremia.

In dogs, the effects of abscesses from the intramuscular injection of a pure culture of staphylococcus were found to be similar to the findings in man. That is, large abscesses developed in the dogs' legs when the injections were made and the plasma iron fell to very low values within forty-eight hours. The maximal anemia did not develop until approximately fifteen days later when the plasma iron had returned to normal and the infection was about healed. There was no rise in erythrocyte protoporphyrin in these dogs. The results were less conclusive with the injection of staphylococcus toxin, although some fall in plasma iron resulted. Typhoid vaccine failed to produce hypoferremia, but the intramuscular injection of sterile turpentine produced both sterile abscesses and marked hypoferremia which was at its lowest value in forty-eight hours. Anemia did not develop following turpentine abscesses except in 3 dogs in whom two successive injections were made.

On the basis of the observations made the authors constructed a working hypothesis for the pathogenesis of the anemia of infection, suggesting that in infection iron is deviated to the tissues and is not available for hemoglobin synthesis. They quoted evidence from the literature to suggest that inflammatory tissue has a peculiar affinity for iron. The serum copper and the protoporphyrin in the erythrocytes are stored awaiting iron availability.

The unavailability of iron was further demonstrated in pigs by a greatly retarded utilization of radioactive iron for new hemoglobin formation in the presence of infection. This was true of both normal animals and those previously rendered iron deficient. The utilization of radioactive iron in pigs on a diet deficient in pyridoxine was likewise greatly retarded but promptly increased after the administration of pyridoxine. In iron deficient pigs turpentine abscesses and one instance of a burn both inhibited the rate of hemoglobin synthesis as measured by the rapidity of utilization of radioactive iron, although in a second burned pig the utilization of radioactive iron was more rapid after the burn than before.

Further studies were made in patients with chronic infection by the continuous intravenous infusion of a solution of ferrous ascorbate. Great difficulty was experienced in maintaining an elevated plasma iron concentration in patients with chronic infection, in contrast to the relative ease in producing a high plasma iron concentration in a patient with simple iron deficiency. These observations were interpreted as indicating that in both normal subjects and in patients with infection, a mechanism, or "brake," exists preventing the rise of the serum iron above certain limits, with a much lower limit in patients with infection than in normals.

Finally, in rats, the fate of injected radioactive iron was studied. In rats with infection, less intravenously or intraperitoneally administered iron was found in the blood and more in the liver than in rats without infection, although the sum of that found in liver and in blood was the same in both groups of animals. In a dog no significant amount of radioactive iron was found in inflamed muscle, the exudate of a sterile abscess, or in the excreta. It was concluded from these studies that in the presence of infection, iron, although diminished in content in the plasma, was not drawn to the site of infection but went largely to the organs concerned with "iron storage," chiefly the liver.

The authors offer no reason for the diversion of the plasma to the liver, but

assume that it is related in some way to the infection. Furthermore they do not believe that the hypoferremia and deviation of the iron to the liver account for the anemia. As the strongest evidence the authors present the fact that prolonged and intensive iron therapy does not bring an increased hemoglobin formation. The presence of excess erythrocyte protoporphyrin and serum copper in these cases eliminates them as limiting factors according to the authors who then suggest that protein may not be readily available for hemoglobin synthesis and may thus be the limiting factor.

THE PTEROYLGLUTAMATES ("FOLIC ACID")

Two years before the synthesis of Lactobacillus casei factor (pteroylglutamic acid) was announced, workers at the University of Arkansas (J. R. Totter *et al.*, *J. Biol.Chem.* **152,** *147 (1944)*) presented evidence that assays for "folic acid" with Streptococcus lactis R did not measure vitamin M activity as determined in experiments with monkeys. These workers noted that this lack of parallelism did not preclude that "folic acid" might be one of two or more substances which possessed vitamin M activity, as indeed proved to be the case. J. R. Totter, V. Mims, and P. L. Day (*Science* **100,** *223 (1944)*) suggested that the determination of "potential" folic acid might be of much greater significance nutritionally than the determination of preformed material. Since the demonstration of the activity of the synthetic pteroylglutamic acid in sprue, nutritional macrocytic anemia, and related syndromes (*Nutrition Reviews* **4,** *11, 13, 15, 338 (1946)*), it has become increasingly important to define clearly those compounds which possess activity for man so that assays of foodstuffs may be carried out to yield information directly applicable to the planning of human dietaries.

It is evident that pteroylglutamic acid is active in sprue, nutritional macrocytic anemia, and related syndromes which are usually classified as deficiency diseases. Evidence for the activity of the fermentation factor or pteroyltriglutamates has been presented by G. A. Goldsmith (*J. Lab. Clin. Med.* **31,** *1186 (1946)*) in nutritional macrocytic anemia, by R. M. Suárez and co-workers (*Ibid.* **31,** *1294 (1946)*), and by E. Jones H. F. Warden, and W. J. Darby (*Ibid.* **32,** *387 (1947)*) in sprue. The activity in man of vitamin Bc conjugate, pteroylheptaglutamate, was also indicated by the data of Suárez *et al.* (*loc. cit.*).

Evidence bearing upon the activity of chemically related substances other than those enumerated above has now appeared (T. D. Spies, R. E. Stone, and R. L. Toca, *South. Med. J.* **40,** *175 (1947)*). These physicians report a patient with pernicious anemia who had previously been treated with parenteral liver extract and pteroylglutamic acid. In the absence of continuous therapy a relapse occurred. The daily oral administration of 20 mg. of synthetic pteroyldiglutamic acid was followed on the fifth day by a reticulocytosis which reached its peak of 25.1 per cent on the eighth day. During the ten days of therapy the erythrocyte count increased from 1.41 million to 2.18 million and the hemoglobin concentration increased from 5.2 g. to 6.7 g. Maturation of the bone marrow occurred during the period of therapy and clinical improvement was reported. The investigators state that a second patient with pernicious anemia in relapse and another with nutritional macrocytic anemia in relapse responded similarly to the administration of this synthetic material. It is of interest to point out that here is an active member of this family of substances which so far has not been demonstrated to occur in foodstuffs.

T. D. Spies and R. E. Stone (*South. Med. J.* **40,** *46 (1947)*) have also reported ex-

periences with some additional related substances. Two patients showed hematopoietic responses to the oral administration of a preparation of vitamin Bc conjugate (pteroylheptaglutamate). The purity of their preparation is not defined. One of the patients had nutritional macrocytic anemia which showed a submaximal response to dosing with 14 mg. of the conjugate per day for nine days. Although a reticulocytosis of 14.7 per cent occurred, there was but little evidence of erythrocyte regeneration—an increase of 0.29 million cells during a fourteen day period. The reported response of a case of pernicious anemia to this material was slight even at a dosage level of 28 mg. per day. Spies and Stone report that pteroic acid was inactive in a case of nutritional macrocytic anemia when injected intramuscularly in doses of 5 mg. per day. This is of interest in that the pteroyl radical is common to all of these members of this vitamin group. Both xanthopterin and glutamic acid likewise proved inactive in pernicious anemia according to these investigators.

Additional qualitative studies of the activities of these various substances over longer periods of time are urgently needed, and this is particularly true of the heptaglutamate. Furthermore, the nutrition worker will hope that quantitative comparisons of the relative activities of these members of the group will be made.

CONVERSION OF TRYPTOPHANE BY MAN INTO N′-METHYLNICOTINAMIDE

Those chemical structures which a given organism requires but cannot synthesize must, of course, be supplied in the diet in the form of vitamins, essential amino acids, etc. The study of the essential nutrients which carry these specific chemical configurations is complicated because in certain instances the organism can utilize either the preformed chemical structure or a precursor which the organism is capable of converting to the requisite form. Thus, certain of the essential amino acids may be successfully replaced in the diet by the corresponding hydroxy acids from which the organism can synthesize the specific amino acids.

An interesting example of a precursor being able to replace a vitamin in the diet occurs with niacin. Ordinarily the rat does not require niacin but on a low protein regimen the urinary excretion of niacin and its derivatives falls markedly. This situation may be reversed by feeding the essential amino acid, tryptophane (*Nutrition Reviews* **4,** *334 (1946)*). Thus, it appears that the rat can convert tryptophane to niacin, a finding which scarcely would have been predicted from the structures of the two compounds.

It becomes immediately important to know whether a similar conversion can occur in man. It has long been inexplicable that a milk diet can prevent or cure pellagra even though the amount of niacin supplied by the milk is little if any greater than the amount present in a pellagra-producing diet. This paradox is possibly explained by the observation that man, like the rat, can convert tryptophane to niacin derivatives. W. A. Perlzweig, F. Rosen, N. Levitas, and J. Robinson (*J. Biol. Chem.* **167,** *511 (1947)*) studied the effect of tryptophane ingestion on the excretion of N′-methylnicotinamide (F_2) and free niacin in the urine of 6 normal, premature infants and 1 full term baby. The basal diet supplied an estimated 0.4 mg. of niacin and 110 mg. of tryptophane daily. On this diet the average daily excretion of N′-methylnicotinamide was 1.5 mg. (0.3 to 3.1). At the same time the unmethylated niacin excreted averaged 0.12 mg. (0.05 to 0.14). Feeding a single dose of 1 g. of l-tryptophane resulted in excretion of an

average of 8.0, 4.7, and 2.8 mg. of N′-methylnicotinamide per child on the first, third, and fourth day following administration. In the few cases where it was measured the unmethylated niacin was found to increase as well but the change was perhaps only twofold instead of fivefold. This clearcut increase in urinary output of the niacin metabolite is of the same order of magnitude as that observed following the ingestion by infants of 100 mg. of niacin (R. A. Coulson and C. A. Stewart, *Proc. Soc. Exp. Biol. Med.* **61,** *364 (1946)*). Ingestion of 2 g. of dl-tryptophane per day for three days resulted in the excretion of as much as 35 mg. of N′-methylnicotinamide per day. (d-Tryptophane was largely excreted in the urine unchanged, hence, only the l-tryptophane could have been responsible for this effect.)

The response to tryptophane was confirmed with 4 adults. Following administration of 5 g. of dl-tryptophane per day for several days, the urinary excretion of N′-methylnicotinamide increased from a control level of 6 mg. per day to a peak of about 15 mg. per day. It seems likely that this represents the availability of at least an extra 50 mg. of the vitamin since only 20 or 25 per cent of large nicotinamide doses are recovered as the methylated derivative.

It will be of at least academic interest to investigate the possibility that intestinal microorganisms may be responsible for the conversion of tryptophane into niacin. In any event, these observations are of major practical significance for human nutrition.

ASCORBIC ACID IN GASTRIC RESECTION FOR PEPTIC ULCER

Despite advances in understanding the significance of ascorbic acid in nutrition, relatively few objective observations have been recorded concerning the role which ascorbic acid deficiency plays in relation to various diseases in man. This is perhaps due in part to the fact that technics for the measurement of ascorbic acid nutrition are not ordinarily available. It is in this light that a study by E. de J. Zerbini (*Arch. Surgery* **54,** *117 (1947)*) is of interest, though it deals with observations on only 2 patients.

Both of the subjects had duodenal ulcer and were subjected to gastric resection. The first, a man of 32 years, had had evidence of duodenal ulcer for sixteen years and had been on a therapeutic regimen which consisted largely of milk and cream with little or no fruit juices or fresh vegetables. This information, however, was not obtained prior to operation nor was it known that the plasma contained no ascorbic acid. During resection of the stomach the tissue was observed to be abnormally friable and capillary bleeding was unusually great. There was a progressive though moderate fall in blood pressure during the latter part of the operation. As the abdomen was being closed, there was a further fall in blood pressure and some depression in respiration, necessitating at one point artificial respiration. Subsequently peripheral circulatory collapse, as indicated by cold and cyanotic extremities, developed. Intravenous injection of plasma and whole blood had little influence on the peripheral circulation or on the blood pressure level. When the patient seemed almost moribund it was learned that neither the plasma nor the white blood cells contained ascorbic acid. Promptly 2 g. of ascorbic acid were injected intravenously. Within a few minutes the blood pressure rose and soon reached normal. The color of the extremities improved and the other evidence of circulatory impairment quickly disappeared. Large amounts of the vitamin were given during the next two days and on the third day the level of plasma ascorbic acid had risen from 0 to 2.4 mg. per 100 ml. Microscopic section of the portion of the

stomach removed at operation showed a pronounced intracellular edema not unlike that seen in scurvy. The patient recovered uneventfully following ascorbic acid therapy and left the hospital on the tenth postoperative day.

The second patient, a man of 42 years of age, had had persistent vomiting and moderate loss of weight and had previously suffered from similar episodes of pyloric obstruction. There was also some bleeding as shown by the passage of several tarry stools. In this case the white cell as well as the plasma ascorbic acid content was 0 and thereupon 3 g. of ascorbic acid were given daily. Following this, the plasma ascorbic acid rose promptly while the white cell content increased more gradually. At operation a prompt fall in the plasma content of vitamin occurred but this was not associated with a significant change in the white cell content. The postoperative course was complicated by the development of pneumonia which responded to chemotherapy but removal of the sutures on the seventh day after operation left a gaping skin wound. At this time the plasma ascorbic acid level was 0.5 mg. per 100 ml. and the white cell content was 30 mg. per 100 ml. The wound ultimately filled in and healed.

That the observations in these 2 patients were probably not coincidental but were really related to ascorbic acid deficiency is suggested by comparison with the observations in the carefully controlled study of J. H. Crandon, C. C. Lund, and D. B. Dill (*New Engl. J. Med.* **223,** *353* (*1940*)) in which it was shown that when ascorbic acid deficiency was severe, the blood pressure fell, and rose to normal only after the administration of ascorbic acid. Since large amounts of ascorbic acid are stored in the adrenal glands and since scorbutic guinea pigs have diminished adrenal cortical hormone (A. Giroud and N. Santa, *Compt. rend. Soc. de biol.* **131,** *1176* (*1939*)), it seems highly probable that the circulatory collapse was the result of ascorbic acid deficiency and that the improvement which followed treatment was due to administration of this vitamin. That wound healing is impaired when ascorbic acid is deficient, is well recognized. It is also worthy of note that T. H. Lanman and T. H. Ingalls (*Ann. Surgery* **105,** *616* (*1937*)) reported that operation or injury increases greatly the need for ascorbic acid and that M. K. Bartlett, C. M. Jones, and A. E. Ryan (*Ibid.* **111,** *1* (*1940*)) found that large concentrations of ascorbic acid are mobilized in areas of injury.

METABOLIC DISTURBANCES IN OSTEOPOROSIS

Osteoporosis, especially that appearing in senility, is often an incapacitating disease, and until recently not amenable to therapy. E. C. Reifenstein and F. Albright (*J. Clin. Invest.* **26,** *24* (*1947*)) present metabolic studies on 12 patients with various forms of osteoporosis and show the effects of several varieties of hormone therapy.

The authors point out that osteoporosis is due to a lack of bone matrix formation and not to failure of mineralization of bone as in osteomalacia. Thus, in osteoporosis, one is concerned primarily with the bone matrix and factors which influence its formation, and destruction will secondarily affect the retention or excretion of calcium and phosphorus.

Eight conditions associated with osteoporosis are listed: disuse atrophy, old age, malnutrition, Cushing's syndrome, the adaptation syndrome of Selye, idiopathic osteoporosis, acromegaly, and the commonest, that found after the menopause.

Five of the patients had postmenopausal osteoporosis, one was a male with senile osteoporosis, 3 showed an acute osteoporotic process following orthopedic opera-

tions, and 3 fell into the classification of Cushing's syndrome.

Calcium, phosphorus, and nitrogen balances were followed. A theoretical nitrogen balance was calculated from the phosphorus balance corrected for calcium balance, assuming that changes in phosphorus balance were due to anabolism or catabolism of intracellular protein. The serum calcium, phosphorus, and alkaline phosphatase were determined at suitable intervals. Urinary 17-ketosteroids are presented for most of the patients.

Estrogenic substances (estradiol and diethylstilbestrol) decreased the calcium and phosphorus excretions in all cases, usually beginning after six days, reaching a maximum in about thirty days, and persisting thirty to fifty days after therapy was stopped. Positive calcium and phosphorus balances were achieved in most instances. Urinary nitrogen likewise decreased and positive nitrogen balance appeared in most instances, although the changes in nitrogen metabolism were not as marked or sustained as that with calcium and phosphorus. Reasons for this discrepancy are not presented by the authors. High control serum phosphorus levels were usual and generally fell during treatment. However, the alkaline phosphatase did not change significantly. Therapy with androgens (testosterone propionate and methyl-testosterone) produced similar changes in calcium and phosphorus excretion and a much more marked decrease in nitrogen excretion with strong positive balances in several instances. No differences were observed between the two forms of estrogens or between the two androgens used. Combined estrogen and androgen therapy produced more striking changes than either alone. Progesterone was without effect.

The authors discuss the clinical use of estrogens and androgens in postmenopausal osteoporosis and conclude that although objective evidence is difficult to obtain, beneficial effects are regularly seen in these patients, both symptomatically and by x-ray. The evidence for retention of calcium and phosphorus, and, to a less extent, of nitrogen in the patients studied supports this conclusion.

BLOOD STUDIES IN RIBOFLAVIN DEFICIENT RATS

Workers at the National Institute of Health, Bethesda, Maryland produced granulocytopenia and anemia in rats by inducing a variety of dietary deficiencies including folic acid, riboflavin, pantothenic acid, and essential amino acids. K. M. Endicott, F. S. Daft, and M. Ott (*Arch. Path.* **40,** *364 (1945)*) described in detail the characteristics of the hematopoietic tissues in folic acid deficiency and the changes which take place during response to folic acid therapy. A further account (K. M. Endicott, A. Kornberg, and M. Ott, *Blood* **2,** *164 (1947)*) represents a comparison of hematopoiesis in riboflavin and in folic acid deficiencies.

A. Kornberg, F. S. Daft, and W. H. Sebrell (*Arch. Biochem.* **8,** *431 (1945)*) in an earlier study showed that rats fed a diet deficient in riboflavin developed granulocytopenia in about 50 per cent of the cases and, less frequently, anemia. The deficiency in white cells responded to treatment with folic acid in 30 out of 33 rats but, curiously enough, responded to riboflavin therapy in only 6 of 28 rats. Riboflavin, on the other hand, alleviated the anemia in 10 of 17 rats while folic acid failed to do so in all of 7 rats. Examination of the tissues of riboflavin deficient rats showed hypoplasia of the bone marrow which was indistinguishable from that seen in rats in which folic acid deficiency had been produced. At the same time, there was atrophy of lymphoid tissue and absence of evidence of blood formation in the spleen.

When folic acid was given to such riboflavin deficient rats the circulating neutrophils in the blood were restored to normal levels in four days and the bone marrow increased in cellularity. This response, however, was less rapid and less complete than the response in folic acid deficient rats. Furthermore, it was noted that if the treatment period was extended for four days, while there was a further response of the hematopoietic tissues, the marked hyperplasia of the bone marrow seen in some of the folic acid deficient rats treated in previous experiments was not observed. After twelve days of treatment there was even some suggestion that granulocytopoiesis might be decreasing to a subnormal level. Evidence of blood formation in the spleen reappeared in both deficiencies after folic acid therapy but was more marked in the group of treated folic acid deficient rats than in the group of treated riboflavin deficient rats. Lymphoid structures also showed some regeneration in the treated group of riboflavin deficient rats. Comparison with folic acid deficient rats could not be made since such observations had not been carried out in the earlier experiment.

These observations, as the authors themselves point out, are somewhat paradoxical. The diet fed the riboflavin deficient rats was adequate if that vitamin was added, and yet, when the vitamin was withheld, signs and symptoms of folic acid deficiency appear which respond to folic acid therapy. It would seem likely that riboflavin deficiency produces this effect by reducing the food intake with resultant protein deficiency. In earlier studies A. Kornberg (*J. Biol. Chem.* **164,** *203* (*1946*)) demonstrated an intimate relationship between protein deficiency and folic acid deficiency although he was unable to explain the underlying disturbance in physiology.

Even more confusing are the observations relating to anemia. In primary folic acid deficiency the bone marrow showed depletion of the erythroid series in granulocytopenic rats whether anemia was present or not. However, in the folic acid deficiency which seemed to be caused by riboflavin deficiency, this was not seen. In rats with granulocytopenia but without anemia, there was depletion of erythroid cells in the marrow. In rats with granulocytopenia and anemia, on the other hand, there was an absolute increase of erythroid cells in the marrow. Furthermore, preliminary experiments have shown that folic acid therapy is without effect upon the anemia but that riboflavin therapy is effective. The authors tentatively assume that the anemia is, therefore, a direct result of riboflavin deficiency. This would seem probable since other workers have found anemia in dogs (H. Spector *et al.*, *J. Biol. Chem.* **150,** *75* (*1943*)), in swine (M. M. Wintrobe, W. Buschke, R. H. Follis, and S. Humphreys, *Bull. Johns Hopkins Hosp.* **75,** *102* (*1944*)), and in monkeys (H. A. Waisman, *Proc. Soc. Exp. Biol. Med.* **55,** *69* (*1944*)) which was associated with riboflavin deficiency.

THE RELATIVE ACTIVITY OF NATURAL AND SYNTHETIC VITAMIN E

Early studies of the relative activity of the purified natural alpha-, beta, and gamma-tocopherols by the rat antisterility test led to the conclusion that the gamma form of vitamin E is about one-twelfth as active as the alpha form and about one-fifth as potent as the beta form (*Nutrition Reviews* **1,** *371* (*1943*)). P. L. Harris, J. L. Jensen, M. Joffe, and K. E. Mason (*J. Biol. Chem* **156,** *491* (*1944*)) continued the investigation by comparing the activity of natural alpha-, beta-, and gamma-tocopherols in pure form, and their crystalline esters with the corresponding dl-tocopherols and esters. Two modifications of the rat antisterility test were used: (a) equal amounts of the tocopherol being tested were given on each of the first ten

days after conception and (b) equal amounts of the tocopherol on each of the fourth, fifth, and sixth days of pregnancy. The synthetic dl, alpha-tocopherol was only 66 per cent as active as natural d, alpha-tocopherol in preventing resorptive sterility in rats. Synthetic dl, beta-tocopherol was about one-half as active as natural beta-tocopherol. The activity relationship of synthetic dl, gamma-tocopherol to natural d, gamma-tocopherol was difficult to establish with tests by the two methods varying from one-tenth to one-half the activity of the natural form.

As a means of extending present knowledge of the physiology of the tocopherols, E. L. Hove and P. L. Harris (*J. Nutrition* **33,** *95 (1947)*) studied the relative activity of the tocopherols in curing muscular dystrophy in rabbits, a condition which would seem to be relatively unrelated to fetal resorption in rats. This was an important investigation not only from the standpoint of comparative physiology but also as a fundamental necessity in the development of a bioassay for tocopherol activity using dystrophic rabbits. The high urinary excretion of creatine during muscular dystrophy rapidly decreases to a normal level when compounds with vitamin E activity are administered. In these experiments creatine and creatinine in the urine were determined daily by the method of O. Folin (*J. Biol. Chem.* **17,** *469 (1914)*) adapted to the Evelyn colorimeter. Creatinuria was expressed numerically as the ratio of the total creatinine (creatine plus creatinine) to preformed creatinine. The response resulting from a test dose of a tocopherol or an ester was taken as the number of days between dosage and the time when the creatinuria ratio reached the same level as at the administration of the test dose. The tocopherol compounds were dissolved in olive oil in such concentration that between 4 and 8 drops from a calibrated pipet delivered the desired test dose. From this procedure natural d, gamma-tocopherol was found to be almost three times as active as the synthetic dl, gamma-tocopherol in curing dystrophy. The natural gamma-tocopherol was 20 per cent as active as the natural alpha-tocopherol and the natural beta-tocopherol about 30 per cent as active as the natural alpha-tocopherol. In general the activity of these forms of tocopherols showed about the same relationship in the cure of dystrophy in rabbits as they did in the prevention of fetal resorption in rats (*loc. cit.*).

Certain differences were found in the activity of the tocopherols and their esters. The tocopheryl phosphate was 35 per cent more active than equivalent doses of d, alpha-tocopherol when both were given orally. When the phosphate was injected intramuscularly, the urinary creatine fell to a normal level much more slowly than after oral tocopherol administration. A slower recurrence of dystrophy was observed with 2 of the 3 rabbits losing weight and dying without developing more than a slight indication of muscular dystrophy. If the recurrence of dystrophy was considered as the point where weight loss began, the activity of the injected tocopheryl phosphate would appear to be from six to fifty times more active than the oral administration of d, alpha-tocopherol.

The interrelationship of the vitamin E requirement for rabbits and the presence of cod liver oil in the ration were demonstrated by C. G. MacKenzie and E. V. McCollum (*J. Nutrition* **19,** *345 (1940)*). The inclusion of the unsaturated fats of tocopherol-free cottonseed oil in the ration of rats has been shown to increase the vitamin E requirement as manifest by fetal resorption (H. Gottlieb, F. W. Quackenbush, and H. Steenbock, *Ibid.* **25,** *433 (1943)*). Hove and Harris (*loc. cit.*) have shown that the tocopherol-free fats of soybean and corn oil and corn oil plus 0.1 per cent hydroquinone were as active as cod liver oil in inducing dystrophy as measured by creatinuria. When the feeding

of the oils was stopped on the sixteenth day, the creatinuria decreased toward normal, but after a temporary recession the creatinuria increased without further feeding of the oils.

These workers expressed the opinion that the cure of dystrophy in rabbits at present did not offer special advantages in the bioassay for vitamin E activity. Even though it is rapid, it is somewhat more laborious and less precise.

Of interest in this research is the observation that the relative activity of the natural tocopherols and their synthetic racemic mixtures are similar for fetal resorption in the rat and muscular dystrophy of the rabbit. Thus two widely different manifestations of vitamin E deficiency in two different species appeared to be dependent upon the same configuration of the molecule and possibly upon the same physiologic processes.

SIMULTANEOUS VITAMIN A AND THIAMINE DEFICIENCIES

The production of multiple nutritional deficiencies in experimental animals has been encountered frequently during the history of nutritional investigation. In the years prior to the development of purified rations which approach adequacy for rodents, the preparation of natural diets which were deficient in only one nutrient was not always possible. Unknown factors were often reduced below optimal levels by the manipulations necessary to reduce the nutrient under immediate investigation to a sufficiently low level to result in the production of deficiency signs. Such complicating deficiencies still are being encountered in investigations with the monkey, guinea pig, and to a lesser extent with fowl. In rodents many nutritional requirements have been defined accurately enough to permit the production of what appears to be a simple deficiency. If desired, it is possible in the case of several nutrients to superimpose one simple deficiency upon a second uncomplicated deficiency. M. Dye, T. Porter, and E. Kelly (*J. Nutrition* **33,** *459 (1947)*) carried out investigations of this type on the effect of simultaneous deficiencies of vitamin A and thiamine. Since the period required for the depletion of the weanling rat is longer for vitamin A than for thiamine, a preliminary period of vitamin A deficiency was necessary prior to the beginning of the thiamine deficient regimen. The criteria for sufficient depletion of vitamin A in rats were a definite plateau in body weight and some evidence of xerophthalmia, both of which were reached at an average of six and a half weeks. At depletion the rats were divided into two series, each of which was composed of a large number of triads of rats, selected by sex, weight, and litter. The series were identical in numbers and distribution of the animals with the exception that the food intake of the rats in the first series was restricted to the smallest amount eaten by any member of the triad, while all animals in the second series received the rations ad libitum. The levels of vitamin A selected for study were 0, $\frac{1}{2}$, $1\frac{1}{2}$, and 3 I.U. per day, combined with 0, 6, and 9 micrograms of thiamine per day at each level of vitamin A. Whenever possible an additional animal receiving 18 micrograms of thiamine per day was included at each level of vitamin A. The experimental period was six weeks during which time daily food consumption and weekly growth records were kept for both series. At the end of the experiment, the surviving animals were chloroformed, autopsied, and gross autopsy findings and animal lengths recorded. There were six triads of rats at each level of thiamine and vitamin A except at those points where 18 micrograms of thiamine per day were studied when fewer rats were available. Differ-

ences in food consumption, weight changes, and length of rat were subjected to tests for significance according to the "t" formula. The criteria used to evaluate the effect of varying levels of thiamine in the diet upon the utilization of vitamin A were food intake, weight change, length of rat attained, and the number of foci of keratinized epithelial tissue which were termed "abscesses" by these investigators.

When thiamine was absent completely from the diet, there were marked losses in body weight regardless of the amount of vitamin A in the diet. When vitamin A was absent from the diet, there were marked but less severe losses in body weight, irrespective of the amount of thiamine in the diet. These two observations indicate that the basal ration was very deficient in both thiamine and vitamin A and that there had been an adequate depletion of the animals in both nutrients.

The ingestion of 6 or 9 micrograms of thiamine per day resulted in a significant increase in food intakes and favorably affected weight changes in all groups, irrespective of the level of vitamin A consumed. As would be expected the weight gains produced were greater in the groups which were allowed the ration ad libitum than in the corresponding groups on controlled food intakes. No difference was observed in the growth effects produced by the 6 and 9 microgram levels of thiamine.

The daily ingestion of $\frac{1}{2}$, $1\frac{1}{2}$, or 3 I.U. in the absence of thiamine did not produce significant differences in the food intake or weight losses of the animals consuming the ration ad libitum. In the presence of thiamine, these amounts of vitamin A did stimulate growth and increased food consumption but not nearly to the extent that was observed for thiamine. The differences again were greater for the animals in the series where the consumption of the ration was not controlled.

No significant difference was observed in the lengths of the animals at the end of the experimental period. There is a possibility not mentioned by these investigators that the rate of growth of these rats which received the high levels of thiamine and vitamin A simultaneously was far below the maximum possible for rats at this age and that the length of those rats may have been inadequate as a measure of the growth stimulating abilities of these two nutrients.

The gross "abscesses" observed at autopsy were most frequent when no vitamin A was present in the diet, regardless of the presence of varying amounts of thiamine. In most groups, the addition of thiamine plus vitamin A appeared to be more effective in the reduction in the incidence of this type of lesion than the administration of vitamin A alone.

From these data it would appear that vitamin A and thiamine each had definite and independent weight stimulating abilities but that the effect of thiamine was three to four times as great as vitamin A under the circumstances of this experiment. A possible synergistic effect of thiamine on the utilization or assimilation of vitamin A was indicated by the results on the incidence of "abscesses." It is a source of speculation as to whether this effect might be better demonstrated by histologic investigation of the development of metaplasia and keratinization of epithelial structures during simple vitamin A deficiency and during vitamin A deficiency complicated with thiamine deficiency. Further data of value might be obtained through similar procedures during recovery from vitamin A deficiency, singly or complicated. The low rate of growth observed in the groups with adequate amounts of vitamin A and thiamine is a point for consideration in the planning of future experiments of this type. It would seem to indicate that the basal ration might well be deficient in factors other than those under immediate consideration.

NOTES

Letter to the Editor

Dear Sir:

I beg to acknowledge with many thanks the receipt of the current numbers of *Nutrition Reviews* . . . they have been placed in our general library where they will be available to other scientific workers and I am asked to convey thanks to you.

I again thank you very much for your sympathy and help indeed in replacing some of our losses suffered during the war.

Awaiting with great interest the receipt of the current issue of *Nutrition Reviews*. . . .

E. J. Sterkin
Professor of Pathological Physiologie
Medical Institute
Dniepropetrousk
USSR

The Vitamin A of Colostrum

D. B. Parrish, G. H. Wise, and J. S. Hughes (*J. Biol. Chem.* **167,** *673 (1947)*) examined the colostrum and also the later milk of a number of cows of four different breeds with regard to vitamin A and carotenoids. Particular interest is in the chemical state of the vitamin—whether present as the alcohol or as the ester, or both.

A chromatographic method for the separation of the alcohol and ester forms of vitamin A was developed, using an adsorbing column of alumina which separated other carotenoid pigments from vitamin A, as well as the two forms of vitamin A from each other.

The data show that about 97 per cent of the vitamin A is present in ester form, both in colostrum and milk, with no significant variation among different breeds. In colostrum, carotene comprised from 89 to 95 per cent of the total carotenoids. Although almost all of the vitamin A in colostrum and milk is in the ester form, preliminary studies indicate that the greater part of the vitamin A in the blood of the cows is in the alcohol form.

Diet During Pregnancy

Bertha S. Burke and Samuel B. Kirkwood of the Harvard School of Public Health have prepared an eight page pamphlet entitled "Daily Diet During Pregnancy." Copies may be purchased from the Department of Maternal and Child Health, Harvard School of Public Health.

Metabolism of Substituted Succinic Acids

The mode of utilization and physiologic effects of branched-chain fatty acids have attracted considerable interest because of (a) the information which might thus be obtained on the intermediary metabolism of fatty acids, and (b) the possible occurrence of such compounds in the synthetic fatty acids prepared by oxidation of hydrocarbons. The latter are a possible source for dietary fats during wartime.

A study of the utilization of various substituted succinic acids by the dog has been made by K. Bernhard and H. Lincke (*Helvet. chim. acta* **29,** *1457 (1946)*). These authors synthesized a number of compounds in which side-chains containing from 6 to 12 carbon atoms were introduced into the succinic acid structure. These neutralized fatty acids were fed daily in small quantities (70 to 300 mg. per kilogram per day, total of 7 to 15 g.) to dogs receiving a stock ration. In general, no physiologic disturbances were observed. The urine was collected quantitatively, acidified, exhaustively extracted with ether, and the extract examined for its organic acid content. Varying amounts of the original acid fed, of succinic acid, and of oxalic acid were recovered from the urine.

The excretion of succinic acid as a result of feeding these substances is of considerable interest. The same phenomenon was observed by R. Emmrich, P. Neumann, and I. Emmrich-Glaser (*Z. f. physiol. Chem.* **267,** *228 (1941)*). The latter authors attempted to explain the excretion of succinic acid by

assuming that the substituted succinic acids inhibited the succinic dehydrogenase of the body, thus leading to the excretion of succinic acid which arose from sources other than the administered fatty acid. W. Franke (*Ibid.* **280,** *76 (1944)*) later showed, however, that no specific inhibition effects on succinic dehydrogenase were displayed by the substituted succinic acids. On the basis of their results, Bernhard and Lincke postulate that those acids with an even number of carbon atoms which cause excretion of succinic acid are first oxidized on the terminal methyl group of the side-chain to the corresponding tricarboxylic acid. This is then degraded by successive beta-oxidations of the side-chain to a 6 carbon tricarboxylic acid which on dehydrogenation yields cis-aconitic acid. The latter is degraded by the known steps of the tricarboxylic acid cycle to succinic acid. Why the succinic acid thus formed should be excreted rather than utilized by the body is not entirely clear, but may result from accumulation of unusually large amounts of cis-aconitic acid. No explanation for the formation of oxalic acid is attempted.

Niacin Metabolism of the Calf

The adult ruminant is commonly assumed to be independent of a dietary source of the vitamin B-complex due to the synthetic activity of the microflora it contains. P. B. Pearson, H. Schmidt, and A. K. Mackey (*Proc. Soc. Exp. Biol. Med.* **40,** *423 (1939)*) and A. H. Winegar, P. B. Pearson, and H. Schmidt (*Science* **91,** *508 (1940)*) reported that sheep can synthesize niacin either in the rumen or in the tissues. An intensive study of this problem, using 2 newborn calves as the experimental animals, has been made by B. C. Johnson, A. C. Wiese, H. H. Mitchell, and W. B. Nevens (*J. Biol. Chem.* **167,** *729 (1947)*). The young calves were chosen because in them the rumen has not yet grown, so that establishment of a microflora was not yet a factor. To further eliminate the possible microflora activity of the rumen during the experiment, a roughage-free diet was used. The absence of bacterial synthesis was indicated by the failure of 1 per cent dietary sulfathalidine to influence growth or niacin excretion during the nine and a half to twelve week period. At autopsy no niacin was found in the rumen or stomach, nor was any food found in the rumen, reticulum, omasum, or abomasum as would occur in the ruminating animal receiving roughage.

The 2 calves grew normally through the twelve week period and showed no deficiency symptoms. As they were fed only a "synthetic milk" virtually free of niacin it was concluded that a dietary source of niacin is not essential for the growth of this species. Niacin and its metabolites were excreted in the urine at a rather constant level throughout the three months of the experiment. On the niacin-free diet about half of the niacin and its metabolites was excreted as N′-methylnicotinamide and half as Lactobacillus arabinosus-active niacin, of which 87 per cent was nicotinamide.

This study suggests that the calf can synthesize niacin in its tissues.

The Specificity of Folic Acid

G. J. Martin, J. Moss, and S. Avakian (*J. Biol. Chem.* **167,** *737 (1947)*) reported that a folic acid-like substance in which the pteridine nucleus is replaced by a quinazoline ring possesses folic acid activity, as shown in the assay procedure developed for folic acid by H. K. Mitchell and E. E. Snell (*Univ. Texas Pub.* **4137,** *36 (1941)*). The new compound was synthesized as a possible "displacing agent" for folic acid, but instead it acted as a growth stimulant. Its action as a folic acid-like growth factor is of particular interest since no other compound had previously been reported which possessed activity similar to pteroylglutamic acid. The authors suggest the probability that a series of molecular configurations exist which possess "folic acid activity."

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The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

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